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# Morning Release of Larvae Controlled by the Light in an Intertidal Sponge, *Callyspongia ramosa*

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**Abstract.** The intertidal sponge *Callyspongia ramosa* releases larvae in the morning under natural light. The photic control of this morning release was studied under experimental light-dark (LD) cycles. Under LD 12:12h cycles (light period, from 6:00 to 18:00), release peaked about 6:00. But the release was not stimulated by the illumination in the morning because the sponge colonies released larvae even in the darkness. The experiments under various light regimes showed that the photic stimulus is not the onset of darkness but, unexpectedly, the onset of light the day before. In fact, *C. ramosa* colonies invariably released larvae about 24 hours after the onset of light under all illumination regimes tested. The tidal cycle and the daily cycle of the seawater temperature did not influence the time of release. Therefore, in nature, the dawning light most likely stimulates larval release on the next day. This photoadaptation in the larval release of *C. ramosa* suggests that the morning release is advantageous for their free-swimming larvae to seek out and settle on the suitable substratum in the intertidal region within their short dispersive period.

## Introduction

Many sponges are phototrophic and some derive at least 50% of their energy requirements from large populations of photosynthetic symbionts, usually blue green algae (Wilkinson, 1983, 1987). Obviously these sponges must occupy a substratum of full sunlight. The free-swimming larvae of such species are probably capable of locating themselves on a habitat exposed to the sun in this dispersive period. On the other hand, the free-swimming larvae of the sponges adapted to the shady habitat must selectively settle themselves on a shaded substratum. The free-swimming larvae of many sponges respond to light (Warburton, 1966; Bergquist and Sinclair, 1968; Uriz, 1982a, b). However, it has not been shown experimentally that the larvae of phototrophic and non-phototrophic sponges show different patterns of behavior in habitat selection (Bergquist *et al.*, 1970; Fell, 1974).

Morning releases of larvae have been reported in several sponge species (Levi, 1951, 1956). *Halichondria panicea* (order Halichondrida) also releases larvae in the morning (Amano, 1986). It was shown that the stimulus for larval release in this sponge is the onset of darkness the evening prior to release, which occurs 15 hours later. In this report I show that *Callyspongia ramosa* (order Haploschlerida) releases larvae in the morning, but its stimulus for release is the onset of light the day before. This is quite different from *H. panicea*. The ecological significance of these photic controls of larval release and of the photoadaptations in the habitat selection of sponge larvae are discussed.

## Materials and Methods

*Callyspongia ramosa* colonies were collected in June from rafts at the Breeding Center of Aomori Prefecture, in northern Japan, about three kilometers from the Asamushi Marine Biological Laboratory where all the experiments were performed. Sponge colonies were collected carefully to minimize damage. They were placed in water-tight containers under water, brought to the laboratory, and transferred into running seawater. Sponges were maintained in clean running seawater to ensure their health throughout the experiments.

In the early morning following collection, colonies releasing many larvae were selected. Larvae filled the mesohyle of such colonies. Usually colonies released larvae for more than ten days in the laboratory. The larvae released from a colony under experimental LD cycles were counted using the method described previously (Amano,

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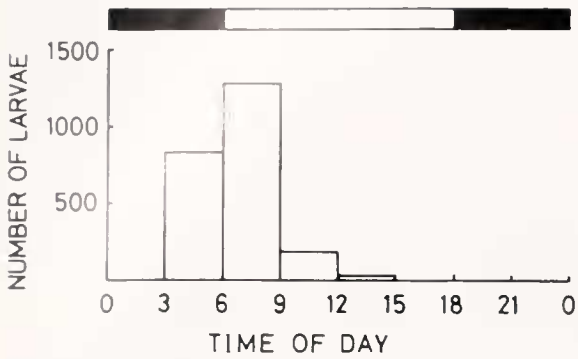


Figure 1. Typical larval release pattern of *Callyspongia ramosa* under LD 12:12h cycles (light period, from 6:00 to 18:00). The colony released largest number of larvae between 6:00 and 9:00, and the numbers decreased thereafter. The arithmetical mean of the release time (MRT) is 6:44. At the top, illumination schedule is shown: the blackened bar indicates the dark period and the white portion, the light period.

1986). Briefly, the colony was fixed with a cotton thread in a photographic developing tank and supplied continuously with running seawater. Thus it could be illuminated or shielded from light at will without interrupting the seawater supply. The released larvae were washed with the outflow, caught by a piece of nylon mesh applied to a plastic vessel, and counted every three hours.

## Results

Although the exact reproductive period of *C. ramosa* in nature is unknown, it released parenchymula larvae in June in the Asamushi Marine Biological Laboratory. The larvae ejected from a osculum swam just below the water surface. The dimensions of a typical larva were about 250 × 150 μm. The larvae were thickly covered with flagella except for a bare posterior pole, and the bare pole was encircled with a band of long flagella. They were dull yellow but contained reddish brown pigments in the posterior pole.

Under natural light, *C. ramosa* released larvae around dawn in the laboratory. This apparent diurnal periodicity was confirmed under artificial LD 12:12h cycles. Figure 1 shows a typical larval release of *C. ramosa* during a 24-hour-period under these conditions. Many larvae were released before 6:00, but most were released between 6:00 and 9:00; the numbers decreased gradually after 9:00. The mean release time (MRT) was arithmetically calculated with these data; the MRT of the larval release shown in Figure 1 is 6:44.

The results in Figure 1 suggest that the onset of light on that morning could not be a photic stimulus to the larval release because many larvae had been released before 6:00 while still dark. This suggestion is shown to be true in Figure 2. A sponge colony that had been under

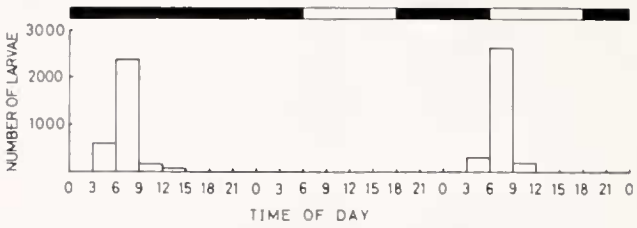


Figure 2. Inhibition of larval release brought about by continuous darkness on the day before. *Callyspongia ramosa* released larvae in the dark on the first day, but did not release on the second day despite illumination from 6:00 to 18:00. MRTs of the 1st and 3rd days are 7:14 and 7:26, respectively.

LD 12:12h cycles was kept in the dark on the first day. On the second and third days it was illuminated under LD 12:12h cycles again. On the first day, the colony released larvae in the dark as if it were still under a LD 12:12h cycle. It did not release on the second day, although it was illuminated from 6:00 to 18:00. This result was not brought about by the loss of release ability of the colony because it released larvae as usual on the third day. These results show that the time of release had been determined by the illumination on the day before. This conclusion is consistent with that of *Halichondria panicea* (Amano, 1986).

Figures 3 and 4 show the results of the experiments designed to determine which photic stimulus, the onset of light or the onset of dark on the day before, triggers larval release. In Figure 3, a colony was illuminated from 12:00 to 18:00: that is putting off of the onset of light by six hours. This putting off of the onset of light delayed the next day's larval release for about 4.5 hours. In Figure 4, a colony was illuminated from 6:00 to 12:00, that is advancing the onset of darkness by six hours. This advance, however, did not significantly advance the time of release on the next day. The delay of the larval release

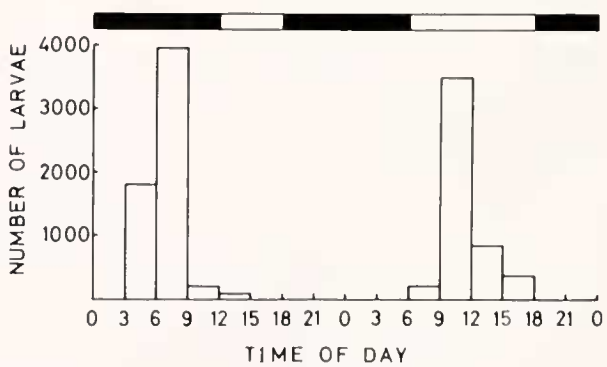


Figure 3. Delay of larval release brought about by six hours' putting off of the onset of light on the first day. The release on the second day was delayed for about 4.5 hours. MRTs of the 1st and 2nd days are 6:47 and 11:18, respectively.

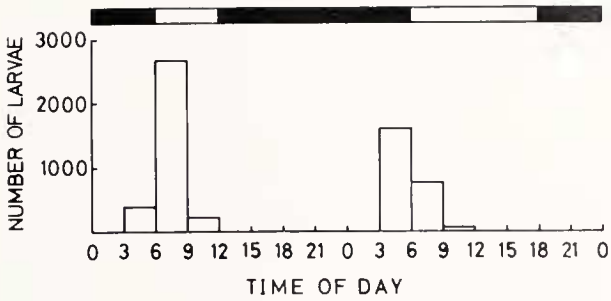


**Figure 4.** Although the onset of darkness was advanced for six hours, the colony released larvae about the same time on the second day as that under LD 12:12 h cycles. MRTs of the 1st and 2nd days are 7:26 and 5:32, respectively.

in Figure 3 was not a result of the shortening of light period because the colony in Figure 4 was also illuminated for six hours. Thus it is clear from these results that larval release was stimulated by the onset of light on the day before. This conclusion is distinctively different from that of *H. panicea* where the onset of darkness was the stimulus (Amano, 1986).

Results of Figure 5 confirm the above conclusion. If the onset of light is truely a stimulus, the time between photic stimulus and larval release must be about 24 hours because *C. ramosa* released larvae at about 6:00 under LD 12:12h cycles (light period, from 6:00 to 18:00). Instead, if the onset of dark were the stimulus, the duration should be about 12 hours. Preliminary experiments showed that a dark-adapted *C. ramosa* colony reacts to illumination for one hour. Figure 5 shows the result of one such experiment where a colony was illuminated from 17:00 to 18:00. Under these conditions it released larvae around 17:00 on the next day, that is, after about 24 hours.

Table I summarizes the results of the experiments presented in this paper and shows the temporal relationships

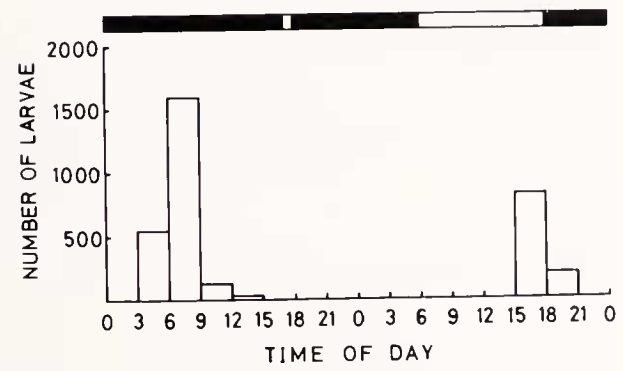


**Figure 5.** Larval release brought about by one-hour illumination. The colony released larvae about 24 hours after the illumination. MRTs of the 1st and 2nd days are 6:59 and 17:04, respectively.

**Table I**

*Constant temporal relationship of larval release to onset of light on the day before under various illumination regimes in* callyspongia ramosa

| Hours from onset of light* | Hours from onset of dark* | Reference |
|---|---|---|
| 24:44 | 12:44 | Fig. 1 |
| 23:18 | 17:18 | Fig. 3 |
| 23:32 | 17:32 | Fig. 4 |
| 24:02 | 23:04 | Fig. 5 |

* Duration between onset time of light or dark and arithmetical mean of release time.

between larval release and the two possible photic signals. Durations between the onset of light on the day before and larval release are constantly about 24 hours under all illumination regimes tested. On the other hand, the onset of dark has variable temporal relationships to larval release. Thus, at least under these experimental conditions, the larval release of *C. ramosa* is brought about by a photic stimulus, that is, the onset of light on the day before.

## Discussion

Under natural illumination, *C. ramosa* releases larvae in the early morning with apparent diurnal periodicity. It is obvious from the results shown in this paper that the larval release is controlled by the light and does not depend on other possible environmental factors. Larval release is independent of the tidal cycle and the daily change of seawater temperature because it showed no temporal relationship to such factors during this study. Besides, it is not controlled by a circadian rhythm because a release peak occurred only once in the continuous darkness. It must be noted, however, that all of the results shown in this paper are based on laboratory experiments. I have yet to study larval release of a sponge in the field; it might be possible in a particular species only. The morning releases of larvae have been reported in several sponge species: *Halichondria panicea* (Amano, 1986), *Haliclona permolis* (Amano, unpub. data), *Oscarella lobularis, Hymeniacidon sanguinea,* and *Halisarca metschnikovi* (Levi, 1951, 1956). Thus the morning release of larvae is likely to be ubiquitous in viviparous sponges.

I have already shown that the onset of light on the day before is the photic stimulus to the larval release of *C. ramosa* (order Haploschlerida) (see Results). In fact, colonies of *C. ramosa* released larvae about 24 hours after the onset of light under all illumination regimes tested (Table I). In nature, the dawning light is probably a stimulus to the larval release of the next day. Therefore *C. ramosa* may release larvae a little earlier even on a dark, cloudy morning if the previous morning was clear. In

*Halichondria panicea* (order Halichondrida), however, the photic stimulus for its larval release is the onset of darkness on the previous day (Amano, 1986). Thus, the onset of light may be a photic stimulus in the order Haploschlerida and the onset of darkness may be a stimulus in the order Halichondrida, although more studies are required to verify this presumption.

Which receives the photic stimulus, a mother sponge or the larvae? The sponge has no ovary; larvae are enveloped by a layer of follicle cells and embedded in the mesohyle. The structure of the follicle and that of the mesohyle seem too simple to receive the photic stimulus and to perform complicated functions associated with larval release. It is more likely that the larvae receive the stimulus directly. Light can reach the larvae through the mesohyle because *C. ramosa* colonies are small and pale in color. When stimulated by the onset of light, mature larvae ready to be released may migrate toward an exhalant canal. Although almost nothing is known about their migration, the larvae must rupture the follicle and move toward an exhalant canal through the mesohyle (Brien and Meewis, 1938; Fell, 1969). *C. ramosa* larvae may require about twenty-four hours to reach the exhalant canal, that is to say, this is probably the lag time before release. To test this hypothesis, the migration of larvae within the mesohyle must be studied in detail during the twenty-four hours following the photic stimulation.

Although sessile animals of aequatic habitat belong to taxa which are very different phylogenetically, they may be grouped together ecologically as producers of dispersive propagules such as planktonic larvae (Sara, 1984). This has been an evolutionary trend, as dispersion and substrate occupation are obviously essential for the success of any sessile animal (Meadows and Campbell, 1972). In the sponge, all viviparous species produce free-swimming larvae as dispersive propagules. It is noteworthy that most intertidal sponges are viviparous and most oviparous sponges inhabit the deeper sea-floor (Reiswig, 1976; Watanabe, 1978; Simpson, 1980). Viviparity appears to be advantageous for the intertidal sponges to occupy a suitable substratum (Bergquist and Sinclair, 1968; Ayling, 1980). If they were oviparous, their eggs and developing embryos might be swept away from the intertidal region by strong currents and waves during a long embryonic period. Free-swimming larvae seem to be able to find and settle promptly on a suitable substratum within the dangerous intertidal region because they respond to light and gravity (Warburton, 1966; Bergquist *et al.*, 1970; Uriz, 1982a, b). The photic controls of larval release shown in this and in a previous paper (Amano, 1986) are probably two instances of the adaptation for efficient dispersion of the swimming larvae of these sessile sponges in the intertidal region.

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